Docket No. 700157-043471-FWC

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	W. Marasco, et al.		
Serial No.:	08/822,033	Group No.:	1632
Filed:	March 24, 1997	Examiner:	P. Woitach
For:	NUCLEIC ACID DELIVERY SYSTEM, METHOD OF SYNTHESIS AND USES THEREOF		

DECLARATION OF DR. WAYNE A. MARASCO

I, Wayne A. Marasco, hereby declare as follows:

1. I am a co-inventor of the above-described application.

2. I am an Associate Professor of Medicine at Harvard Medical School, and physician at Dana-Farber Cancer Institute in the Department of Cancer Immunology and AIDS. A copy of my CV is attached hereto.

3. I am aware of the Office Action issued on February 11, 2003 in the above-described matter and am familiar with the references discussed in that Office Action.

4. The Examiner relied upon Beug et al. (Canadian Patent Application 2,012,311), Chaudhary et al. (*Proc. Natl. Acad. Sci. USA* 87:1066-70 (1990)), Wu and Wu (U.S. Patent No. 5,166,320), and Ryder et al. (*Proc. Natl. Acad. Sci. USA* 86:1500-03 (1989)).

5. I do not believe that these references in any way teach or suggest our method of nucleic acid delivery to a desired target cell. The method involves using a fusion protein prepared by recombinant techniques containing an antibody targeting moiety and a nucleic acid

binding moiety to specifically deliver the nucleic acid to a desired target cell. This delivery system can deliver any desired nucleic acid.

6. Chaudhary in no way teaches a nucleic acid delivery system. No nucleic acid is delivered to the target cell. Chaudhary was directed to an *improved cloning* technique, as reflected in its title: "A **rapid method of cloning** functional variable-region antibody genes in *Escherichia coli* as single-chain immunotoxins." [emphasis added]

7. Moreover, the "immunotoxins" of Chaudhary that are delivered to a specific cell are in the form of a protein. This raises all sorts of problems with antigenicity against the Chaudhary fusion protein that do not occur with the present system.

8. Consequently, nothing in Chaudhary suggests that its fusion proteins could be useful for delivering a nucleic acid to a cell.

9. Wu describes a three-component system for delivering nucleic acids into a cell. This system is summarized in the paragraph bridging columns 3 and 4 of Wu. The first component is the nucleic acid for delivery, referred to as a "polynucleotide" or "PN". The second component is a "receptor-specific ligand" to allow cell targeting. This second component is linked to the "PN" via a third component, called the "PN-binding component."

10. However, there are several critical differences between the Wu chemical conjugate and our fusion protein. First of all, the cell targeting moiety and the DNA binding moiety of our invention are joined through a peptide bond, whereas Wu uses a disulfide bond, i.e. a chemical covalent bond.

11. Another important difference between our fusion protein system and the Wu system is the nature of the nucleic acid binding moiety. In our system, this moiety is a nucleic acid binding protein which is expressed as part of a fusion protein. In contrast, in the Wu

system, there is nothing that suggests that the nucleic acid binding moiety can be expressed as part of a fusion protein with the receptor-specific ligand. Given the challenges to cloning at the time, as reflected in Chaudhary, it would not have been obvious to the typical researcher in this field at the time to adopt Wu's system to create a fusion protein. Indeed, it would be taught against, since Wu taught that it was beneficial that the covalent bond could be cleaved in the cell whereas a peptide bond is not readily cleaved (Col. 5, lines 37-48).

12. Yet another critical difference between Wu and our invention, is that our system is reproducible, not transient. Our system only requires an initial cloning step to create the gene encoding the fusion product; once that construct is generated, it does not have to be re-created for each administration, unlike a chemical conjugate.

13. Beug describes a system for delivering a nucleic acid to a cell which is basically the same as the Wu system. Beug uses a soluble transferrin/polylysine/DNA complex to transfer the DNA into a cell, by interaction of transferrin with its receptor on the surface of the cell. Moreover, Beug focuses exclusively on the use of transferrin, not an antibody.

14. Another difference between Beug and our invention is that because Beug is interested in a chemical conjugate, Beug teaches that the ratio of transferrin to the polycation can range from 10:1 to 1:4, or even to further ratios. (See the paragraph bridging pages 5 – 6). However, our fusion protein necessarily has a strict 1:1 ratio of the antibody and the DNA binding moiety.

15. There is nothing in the combination that suggests over method of using recombinant means to deliver the nucleic acid to a desired cell. However, even if one did assume for the sake of argument that it would have been obvious to make a recombinant fusion protein, one would have expected it to behave identically to the chemical conjugate.

16. However, as discussed below the two types of delivery systems unexpectedly do not behave the same.

17. Our surprising discovery, as reported in Li et al. ("Single-chain antibody-mediated gene delivery into ErbB2-positive human breast cancer cells" in *Cancer Gene Ther.* 8: 555-565 (2001)), is that the fusion protein is importantly more selective in its targeting than the same components chemically joined. In Li, we compared the expression of a nucleic acid delivered to a cell using both of the systems: the fusion protein approach (Figure 6C) and the chemical conjugate approach (Figure 7B).

18. Figure 6C of Li shows that the cellular uptake of the fusion protein is 8 – 10 fold more selective for cells containing the target, ErbB2 receptor (SKBR3 cells), than for cells which do not express this receptor (the MCF7 cells), as determined by expression of the nucleic acid delivered via the fusion protein. The results for the analogous experiment with the chemical conjugate are shown in Figure 7B. Here, it is clear that the cellular uptake for the conjugate is only 4 – 5 fold more selective. Thus, the fusion protein approach offers a significant improvement in the selectivity of uptake.

19. I respectfully disagree with the Examiner's interpretation of these results. The focus of our work at the time of this application was filed was to find methods for **selective delivery** of a nucleic acid to a specific cell type of interest. Instead, the Examiner has indicated that the focus was on "increasing uptake of the complex in the target cell." However, efficient means for generally delivering nucleic acids into cells were already known at the time, including for example standard calcium phosphate transfection and lipofection. Our goal was to find methods for selective delivery hence the use of an antibody that would target desired cells. Obtaining that goal is what the results of Li confirm.

20. In Li, our comparison of the two approaches discussed here, fusion proteins and chemical conjugates, clearly illustrates the unexpected superiority of the fusion protein strategy. In fact, the difference may be even greater than it appears in our initial results as reported in Li, because the chemical conjugate approach we used included the transfection reagent Dotap. This liposomal formulation is used for the transfection of DNA and RNA, and is expected to generally increase the efficiency with which a cell takes up a nucleic acid. Thus, it is likely that the Dotap increased the uptake of the reporter DNA resulting in an apparently higher uptake for the chemical conjugate over the fusion protein.

21. Accordingly, I respectfully believe that our system for delivering a nucleic acid to a cell by fusing an antibody to a nucleic acid binding moiety, and the unexpectedly better selectivity our system affords, is in no way suggested by the prior art references.

22. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

6-11-03
Date

Wayne A. Marasco